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BIODEGRADATION AND TRANSFORMATION OF NITROAROMATIC COMPOUNDS POSTPRINT (Code 20)

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The addition of a nitro group to aromatic compounds dramatically alters the chemical properties of the molecule, and consequently the biodegradation pathways evolved by bacteria for the assimilation of the unsubstituted aromatic compounds are not capable of accommodating the corresponding nitro compounds. Few natural nitroaromatic compounds are known, and release of synthetic compounds into the biosphere has been a relatively recent event. Even so, microbes have evolved a variety of strategies for metabolism of aromatic nitro compounds.

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Biodegradation and Transformation of Nitroaromatic Compounds

SHIRLEY F. NISHINO AND JIM C. SPAIN

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The addition of a nitro group to aromatic compounds dramatically alters the chemical properties of the molecule, and consequently the biodegradation pathways evolved by bacteria for the assimilation of the unsubstituted aromatic compounds are not capable of accommodating the corresponding nitro compounds. Few natural nitroaromatic compounds are known, and release of synthetic compounds into the biosphere has been a relatively recent event. Even so, microbes have evolved a variety of strategies for metabolism of aromatic nitro compounds.

METABOLISM AND PATHWAYS

Recently, a variety of novel oxidative and reductive mechanisms that lead to the complete mineralization of nitroaromatic compounds have been discovered. Extensive reviews of the recent research in these areas have been published (27, 46, 68, 69) and will be discussed only briefly here. What has emerged from this work is a dichotomy of how microbes respond to the presence of nitroaromatic compounds and how specific organisms can be isolated or exploited for their abilities to transform nitroaromatic compounds. In one scenario, the nitro compound is degraded (mineralized) and provides the organism with a source of carbon, nitrogen, or energy; therefore, enrichment and selection for microorganisms can be based on the ability of the nitro compound to support growth. In another scenario, there are other nitroaromatic compounds that will not support growth of microorganisms and thus provide no selective advantage that can be exploited for the isolation of organisms able to degrade the compounds. Nevertheless, microorganisms can often transform the aromatic nitro compounds through fortuitous reactions of nonspecific enzymes. These types of reactions are particularly common among the fungi and anaerobic bacteria. Although these transformations do not result in the complete removal of toxic compounds, novel strategies of bioprocess engineering offer the potential for use of biotransformation systems for the complete degradation or detoxification of nitroaromatic compounds.

Fungi

Various fungi, but in particular the white and brown rot fungi, under low-nutrient conditions produce extracellular ligninolytic enzymes that attack both the phenolic and nonphenolic components of lignin (21). The nonspecific nature of these systems allows the same enzymes to transform a wide variety of other substances, including nitro- and aminoaromatic compounds (5). The white rot fungus, *Phanerochaete chrysosporium*, has in recent years been found to degrade 2,4-dinitrotoluene (78) and 2,4,6-trinitrotoluene (TNT) (22, 49) under ligninolytic conditions. However, the ligninolytic system does not appear to be involved in the initial reduction reactions, which require the presence of live mycelia, but the ligninolytic enzymes are necessary for the subsequent reactions that lead to mineralization of the amino intermediates.

Anaerobic Bacteria

There have been several reports of degradation or transformation of nitro and aminoaromatic compounds under anaerobic conditions. Gorontzy et al. (28) found only nonspecific reduction of the nitro group to the corresponding amine by a variety of methanogenic bacteria, sulfate-reducing bacteria, and clostridia. None of the 17 strains examined could utilize the nitrophenols or nitrobenzoates as growth substrates, and the authors concluded that reduction of the nitro group was a nonspecific detoxification mechanism for those organisms. In contrast, sulfate-reducing bacteria use 2,4-dinitrophenol, 2,4- and 2,6-dinitrotoluene (8), and TNT (7, 59) as sources of nitrogen. The nitroaromatic compounds are reduced to the corresponding amines, whose subsequent fate is not clear, although reductive deamination has been proposed (8) as a possible mechanism for the elimination of the amino groups. Reductive deamination of 4-aminobenzoate metabolites before ring cleavage (64) and degradation of 3-aminobenzoate (65) have been demonstrated under anaerobic conditions. Nitroreductase activities in clostridia (47, 59, 60) produce nitroso-, hydroxylamino-, and aminoaromatic products from the parent nitroaromatic compounds.

Aerobic Bacteria

Aerobic bacteria can degrade nitroaromatic compounds through a variety of oxidative or reductive pathways. The known pathways for degradation of nitroaromatic compounds involve either the removal or the reduction of the nitro group and conversion of the resulting molecule into a substrate for oxidative ring fission. The cleavage products can be readily converted into the dicarboxylic acids of intermediary metabolic pathways.

Reductive pathways generally involve the conversion of

the nitroaromatic compound to hydroxylamino- or aminoaromatic intermediates (48). The reduced intermediates may be further transformed before ring cleavage and liberation of the nitrogen as ammonia. The reduction products are often the same as those produced by anaerobic bacteria (12), but anaerobic conditions are not necessary. The known transformations of the hydroxylamines include the action of a hydroxylaminolyase which converts hydroxylaminobenzoate to protocatechuate (31, 33, 62) and of hydroxylaminobenzene mutase, which converts hydroxylaminobenzene to 2-aminophenol (54). A novel mechanism that results in the release of nitrite from the rearomatization of a reduced hydride-Meisenheimer complex has been demonstrated for picric acid (44) and TNT (81).

Oxidative catabolism of nitroaromatic compounds can be initiated by the action of either monooxygenase or dioxygenase enzymes. Monooxygenases catalyze the replacement of the nitro group by a hydroxyl group in the degradation of 4-nitrophenol (73), 2-nitrophenol (86), and 4-methyl-5-nitrocatechol (32), resulting in the liberation of nitrite. Dioxygenases catalyze the initial attack on 2,4-dinitrotoluene (74), 1,3-dinitrobenzene (53), nitrobenzene (55), 3-nitrobenzoate (51), and 2-nitrotoluene (3, 34). All of these oxidative reactions result in the release of the nitro group as nitrite and the formation of dihydroxy aromatic compounds able to serve as substrates for ring fission and subsequent metabolism.

ISOLATION (SELECTIVE ENRICHMENT) OF SPECIFIC BACTERIA ABLE TO GROW ON NITROAROMATIC COMPOUNDS

The current understanding of specific mechanisms for the degradation of nitroaromatic chemicals has been facilitated by the isolation of bacteria able to use nitroaromatic compounds as growth substrates rather than as nonspecific electron acceptors. Selective enrichment (38) to increase the relative abundance of bacteria with desired degradative abilities is the first step in this process. Factors to be considered prior to the initiation of an enrichment culture include the source of the inoculum, the concentration of the nitroaromatic compound, selection strategies for isolates, and methods to detect growth of the culture or metabolism of the nitroaromatic compound.

Inoculum

Source of Inoculum

Traditional strategies involving selection from activated sludges or a variety of ecosystems have not been useful for isolation of bacteria that degrade the more recalcitrant nitroaromatic compounds. Bacterial strains that use specific nitroaromatic compounds have been selected primarily from inocula previously exposed to the compound. Bacteria from a variety of sources can degrade compounds such as 4-nitrophenol that have been widely distributed in the environment (1, 37, 72, 82). In contrast, bacteria which mineralize the more recalcitrant 2,4-dinitrotoluene have been isolated only from 2,4-dinitrotoluene-contaminated sites (56, 74). Nitrobenzene-degrading strains can be readily isolated from nitrobenzene-contaminated sites but not from uncontaminated sites (55). A similar result was observed for strains able to degrade s-triazines (14). These observations suggest that the presence of xenobiotic compounds provides the selective pressure for the development of the ability to degrade specific contaminants.

Adaptation to Degrade Nitroaromatic Compounds

Microbial communities can often adapt (acclimate) to degrade novel organic compounds. That is, the rate of degradation of a compound is increased by exposure of the microbial community to the particular compound (71). The mechanisms subsumed under the term "adaptation" are numerous but can be classified into three general categories: (i) growth of a small population that is capable of utilizing the compound, (ii) delayed induction of enzymes involved in the catabolic pathway, and (iii) genetic change(s) that enables the microorganisms to grow at the expense of the chemical. The mechanism involved in the adaptation of any particular microbial community to a novel substrate can be suggested by the length of the lag time from the initial exposure of the population to the chemical to the start of rapid disappearance of the chemical (67). Short lag periods of hours to a few days suggest that induction of a degradative pathway is the only requirement for the population to be able to degrade the compound. Lag periods of intermediate length, showing a gradual increase in rate of degradation, suggest that the lag period is the time required for the growth of a specific population of organisms capable of degrading a specific compound. Finally, extended lag periods followed by abrupt increases in degradation rate suggest that a genetic change allowed the microbial community to use the organic compound as a growth substrate. Support for the third mechanism is provided by the observation that nitro- and chloroaromatic compounds previously thought to be resistant to biodegradation are now readily isolated from sites contaminated with such compounds but not from pristine sites.

Substrate Concentration

Careful attention must be given to the concentration of the nitroaromatic compounds available to the microbial population. Choice of an appropriate substrate concentration for selective enrichment involves consideration of toxicity, the concentration necessary to support growth, and the solubility of the nitroaromatic compound in the growth medium.

Toxicity

Toxicity both of the parent compound and of possible metabolic products must be considered. Nitroaromatic compounds typically serve as growth substrates for microorganisms at concentrations ranging from 10 to 100 mg/liter; however, some are toxic in that range as well. Concentrations in the lower part of the range may provide little selective pressure for isolation of bacteria able to degrade nitroaromatic compounds and may be difficult to measure; even so, initial concentrations of 10 to 20 mg/liter have been used successfully. When the initial amount is metabolized, the concentration of substrate supplied in subsequent additions or transfers can be increased until toxicity is exhibited. A lengthened lag period or slower rate of utilization can be taken as a sign of toxicity. If information about the concentrations of contaminants in the ecosystem chosen as a source of inoculum is available, it is often a good strategy to use a concentration of substrate similar to that found at the edges of the contaminated zone. If the concentration of substrate supplied is not enough to support extensive growth, the substrate can be added repeatedly.

Availability

With nonpolar nitroaromatic compounds, the solubility of the compound in aqueous solutions may be too low to support growth. A recent strategy successful with several synthetic compounds has been the use of dual-phase systems,

in which the substrate of interest is dissolved at a high concentration in an inert organic solvent. The solvent serves as a reservoir of substrate, which is released to the aqueous phase as the substrate is utilized by the bacteria. A variety of solvents have been used with different levels of success (13, 19, 52, 61, 76). To date, this approach has not been used extensively with nitroaromatic compounds.

Procedures for Isolation

Initial Selection Conditions

The initial selection of cultures able to degrade nitroaromatic compounds generally is done in batch cultures. One gram of soil or 1 to 10 ml of water inoculated into 100 ml of a minimal medium supplemented with the appropriate nitroaromatic substrate as the sole source of nitrogen or carbon is a good starting point. The culture should be monitored for degradation of the nitroaromatic compound, and transfers should be made at appropriate intervals before the substrate is completely removed. As the nitroaromatic compound is degraded, a portion of the culture should be diluted 10-fold with fresh medium, and the process is repeated. If the degradative capability persists through multiple transfers, the inoculum size can be gradually decreased to increase the selection pressure and dilute out additional nutrients and substrates present in the initial inoculum. A variation on the batch culture technique is continuous perfusion and recirculation in small soil columns (4).

Isolation of Degradative Strains

When a mixed culture with the desired metabolic capability is available, individual strains can be isolated directly from the enrichment culture, or the enrichment culture may be placed in a chemostat to increase the selection pressure for the desired degradative capability. Individual strains can be isolated by spreading samples of the culture onto agar plates supplemented with the nitroaromatic compound or onto a complex medium such as nutrient agar. If the original inoculum came from an oligotrophic environment, use of diluted media such as $\frac{1}{10}$-strength nutrient agar will often be more successful than use of a full-strength rich medium. Isolation on complex media is useful when bacterial growth is very slow on minimal media or when the transformation of the nitroaromatic compound is only partial. Yeast extract (5 to 20 mg/liter) can be added to minimal media at this stage if the isolate seems to require growth factors. Individual colonies that grow on any of the plates are then tested for degradative ability. Testing of metabolic capabilities can be accomplished by auxanography (58) or by use of gradient plates (84).

Enhancement of Degradative Abilities

Enrichment cultures or isolated strains can be subjected to additional selection to enhance degradative abilities. Growth in chemostats at high dilution rates can be used to provide a continuous strong selection pressure for the desired degradative ability (36, 41). Various means of chemical or transposon mutagenesis (20) might also result in improved degradative capability. Molecular approaches to strain construction can be used to improve or expand the catabolic abilities of degradative strains (77). Strain construction in combination with chemostat selection has recently proved useful in isolating a *Pseudomonas* strain capable of mineralizing TNT (18).

Substrate Delivery

The volatility and water solubility of the nitroaromatic compound determine the means of delivery. Substrates that are readily soluble in aqueous solutions at concentrations useful for culturing bacteria can be dissolved directly in the culture medium. Such substrates include the isomeric nitrophenols, nitrobenzene, 2,4-dinitrotoluene, 1,3-dinitrobenzene, and TNT. The latter three nitroaromatic compounds, although soluble at the final working concentration, may be slow to dissolve and can be dissolved in a carrier solvent such as methanol, ethanol, acetone or dimethylformamide before addition to the culture medium. The nitroaromatic compound can also be dissolved in a volatile carrier such as acetone, and the resulting solution is used to coat the bottom of the culture vessel. The solvent is allowed to evaporate before addition of the culture. Volatile substrates may also be added to a culture in the vapor phase. For example, nitrobenzene can be provided to individual petri dishes by placing a cotton-plugged Durham tube containing nitrobenzene into the lid of the inverted agar plate, which is then sealed (26). Volatile substrates can also be provided by placing the plates into a sealed enclosure such as a desiccator with a small open container of the nitroaromatic substrate. Volatile substrates can be supplied to liquid cultures in the gas stream used to provide aeration. A single crystal of slightly water soluble compounds such as the nitrotoluenes can be placed in the center of a previously inoculated agar plate to produce a substrate gradient. Dual-phase cultures should be considered if high concentrations of a relatively insoluble nitroaromatic compound are required.

Nitroaromatic Compounds as Nutrient Sources

Nitroaromatic compounds may be used as either carbon or nitrogen sources. The decision as to whether to use the substrate as the sole source of carbon, nitrogen, or both may depend on the toxicity or solubility of the nitroaromatic compound. If the compound is used as the sole source of nitrogen, a source of carbon must be supplied in nitrogen-free minimal medium (10). A variety of easily metabolizable carbon sources such as glucose (9, 80), acetate (16, 42), succinate (10), lactate (2), glycerol, and pyruvate have been used. It has been suggested that an array of carbon sources be present in nitrogen-limited cultures so as not to limit the range of organisms able to grow in the culture (14); alternatively, multiple cultures, each with a different carbon source, can be inoculated.

In some instances the nitroaromatic compound can serve as the sole carbon and nitrogen source (51, 54, 55, 74). If there are multiple nitro groups on the aromatic ring, the elimination of nitrite can lead to toxicity. For example, during degradation of 2,4-dinitrotoluene by *Burkholderia* (formerly *Pseudomonas*) sp. strain DNT, growth is inhibited when nitrite concentrations approach 1 mM (56).

If bacteria have growth requirements other than the nitroaromatic compound, the addition of vitamins, amino acids, or more complex mixtures such as yeast or soil extracts might be necessary. Such factors must be supplied at levels that do not cause catabolite repression (23), which is expressed as increased lag times before growth on the nitroaromatic compound. Glucose, acetate, and succinate are frequent catabolite repressors because of the ease with which they are metabolized. Alternative substrates such as arginine, glycerol, or specific vitamins are less likely to act as catabolite repressors.

Detection of Degradation of Nitroaromatic Compounds

Substrate Disappearance

Substrate disappearance is a primary indication that the nitroaromatic compound is being utilized. High-performance liquid chromatography (HPLC) with UV detection is most commonly used to monitor substrate disappearance in liquid cultures. Capillary electrophoresis shows considerable promise for identification and quantitation of nitroaromatic compounds (57), but it is less sensitive than HPLC. Many nitroaromatic compounds are colored, so a visible decrease in the color of the culture can indicate substrate utilization. However, the color of some nitroaromatic compounds, such as nitrophenol, is pH dependent, and a slight shift in pH can give the appearance of substrate utilization. If the substrate is volatile, gas chromatography may be a convenient analytical method. However, disappearance of the substrate is only presumptive evidence that the compound is used for growth, and both uninoculated and killed controls must be included to ensure that sorption, volatilization, photolysis, or instability of the compound in aqueous solution is not confused with biodegradation. Disappearance of ^{14}C-labeled substrates indicates biodegradation only if a significant portion of the radiolabel is released as $^{14}CO_2$ and only if the fraction of the radiolabel in $^{14}CO_2$ is much larger than the level of radiochemical impurities.

Growth

If the nitroaromatic compound is used as a source of carbon or nitrogen, growth will accompany the disappearance of the substrate. Growth is easily recognized by increases in optical density in liquid cultures or by increase in colony size on agar plates. Growth can also be detected by increased protein concentration. An increase in cell counts is not reliable, because one of the possible starvation responses is an increase in cell numbers (50), that is, cell division without an increase in biomass. However, if cell numbers increase several orders of magnitude in the presence of the nitroaromatic compound but not in its absence (control cultures), then such increases can be taken as evidence of growth provided that cell yields are consistent and proportional to substrate disappearance.

Metabolite Accumulation

Metabolite accumulation can indicate that the nitroaromatic compound is a growth substrate and also provide insight into the degradative pathway. Steady accumulation of a metabolite indicates that it is a dead-end product and not part of a productive catabolic pathway. Transient accumulation of a metabolite during the early stages of culture growth suggests that the enzymes of the catabolic pathway are not induced simultaneously and that the metabolite is an intermediate of the pathway. Metabolite accumulation may be accompanied by either disappearance of a colored substrate or appearance of a colored intermediate, or both. For example, 2,4-dinitrotoluene is colorless in aqueous solutions, but the first metabolic intermediate produced by *Burkholderia* sp. strain DNT, 4-methyl-5-nitrocatechol, is bright yellow (74). Sequential appearance and disappearance of the metabolite during induction of *Burkholderia* sp. strain DNT on 2,4-dinitrotoluene turns the culture fluid from colorless to yellow to colorless again. Color changes or the lack of them can also be misleading. Two pathways for the degradation of 4-nitrophenol are known; one involves monooxygenation to hydroquinone (70), and the other involves dioxygenation to 4-nitrocatechol (39). Both 4-nitrophenol and 4-nitrocatechol are yellow; hydroquinone is colorless. Persistence of the yellow color in a culture containing 4-nitrophenol can indicate either that 4-nitrophenol is not degraded or that it has been converted to 4-nitrocatechol.

Ammonia and Nitrite Release

The known aerobic pathways for degradation of nitroaromatic compounds all result in the release of the nitro group as either ammonia or nitrite. The appearance of either of these in a culture would indicate the degradation of the nitroaromatic compound. To date, all pathways that result in the release of nitrite from a nitroaromatic compound, with the exception of the picric acid catabolic pathway (44), involve the oxidative release of all the nitro groups prior to ring cleavage. Catabolic pathways that result in the reduction of the nitro group can release the ammonia before or after ring cleavage. Release of these metabolites is concomitant with catabolism of the nitroaromatic substrate. Bacteria able to assimilate or transform nitrite or ammonia can mask the release of these metabolites from the nitroaromatic substrate.

Analytical Methods

Many of the analytical methods mentioned above are discussed elsewhere (25). HPLC is usually performed on C_{18} or C_8 reverse-phase columns with mobile phases consisting of mixtures of water and a less polar solvent such as methanol, acetonitrile, or tetrahydrofuran. Simple linear gradients from a more polar to a less polar mobile phase will separate many nitroaromatic compounds and their metabolites. Ion pair chromatography is often used to separate amino compounds with isocratic mobile phases. Common ion pair reagents such as tetrabutylammonium hydrogen sulfate and hexane sulfonic acid are available in commercial formulations. Photodiode array detectors offer a great improvement in convenience over variable-wavelength UV-visible light detectors. There are many simple but sensitive colorimetric assays for analysis of ammonia and nitrite (15). A commercially available assay for ammonia based on reductive amination (Sigma) is useful when metabolic intermediates interfere with standard colorimetric assays.

DESIGN AND OPERATION OF BIOREACTORS

Widespread environmental contamination by nitroaromatic compounds and explosives has created much of the impetus for recent research on their biodegradation. The best currently available technology for treatment of such materials involves incineration of soils or sorption to activated carbon, which is in turn incinerated. Incineration is a very costly treatment technology and draws much public criticism. Accordingly, much effort has been directed toward developing bioremediation systems, many of which include the use of bioreactors as a key component of the treatment system. All of the treatments described in this section involve metabolism of the nitroaromatic compound by microorganisms unable to use them as growth substrates. Therefore, they require additional sources of carbon and energy for growth and maintenance of the microbial cultures.

Composting

In its simplest form, the process of composting often does not require an actual container and is therefore easily ex-

pandable to accommodate large volumes of contaminated materials. It does, however, require large amounts of materials handling, space to accommodate bulk matter, and containment of leachates. Generally, not more than 10% of the composting pile can be contaminated soil (66); the rest is made up of compostable organic matter. Composting has been demonstrated for decontamination of soils contaminated with TNT and other explosives (83). Chemical and toxicological tests of composted explosive-contaminated soils indicate large reductions in the concentrations of explosives and their metabolites and the toxicity of leachates after composting (29, 30), but it is still not clear that acceptable levels of toxicity and mutagenicity are reached in composted soils. The ultimate fate of the nitroaromatic compounds in the residue is not known, and the volume of the hazardous material is increased considerably.

Anaerobic Treatment Systems

Anaerobic treatment systems have been proposed as a means of avoiding the accumulation of partially reduced intermediates during degradation of TNT. Under strictly anaerobic conditions ($E_h \leq 200$ mv), TNT can be completely reduced to triaminotoluene (59). Anaerobic treatment of explosive (primarily TNT)-contaminated (24) and herbicide (dinoseb)-contaminated (40) soils has been demonstrated in open bulk containers of soil, phosphate buffer, and potato starch. The potato starch served as a readily degradable carbon source which allowed the rapid establishment of anaerobiosis. In the case of the herbicides, no aromatic compounds remained in the bioreactors; however, with TNT, cresols and small organic acids remained as end products (24). To date, this is the only commercially available system specifically developed for bioremediation of nitroaromatic compounds.

Anaerobic/Aerobic Systems

To eliminate the hydroxytoluenes or aminotoluenes remaining in the anaerobic bioreactors following the disappearance of TNT, second-stage aerobic reactors have been proposed to hasten the removal of those intermediates which are more rapidly degraded under aerobic conditions (24, 63). Upon aeration, triaminotoluene bound to soil undergoes an oxidative polymerization, which immobilizes the chemical (63). However, further research is required to demonstrate whether the immobilization is permanent and whether any residual toxicity remains.

A two-stage anaerobic-aerobic process for degradation of nitrobenzene has also been described (17). Although nitrobenzene can be mineralized aerobically, there is potential for losses of nitrobenzene through air stripping. This problem can be avoided through a process which is initiated by a nonspecific anaerobic reduction of nitrobenzene to aniline, followed by aerobic degradation of the aniline. Glucose can serve as the carbon source and hydrogen donor for the anaerobic phase.

Fungus-Based Remediation Systems

The nonspecific, extracellular enzymes of the white rot fungi make them attractive candidates for bioremediation systems. Ligninolytic cultures of *P. chrysosporium* have been grown in a bench-scale fixed-film silicone membrane bioreactor (79) to study the potential for degradation of TNT (11). TNT in wastewater from munitions plants has also been successfully degraded by *P. chrysosporium* immobilized on a rotating biological contactor (75). Other proposals include the combination of fungi with bacteria in systems in which the fungi would first detoxify or modify the xenobiotic compound so that the bacterial population could mineralize the resulting metabolites (5).

Slurry-Phase Systems

Slurry-phase bioreactors in which soil is kept in suspension by mechanical mixers are being evaluated as a means of bioremediating contaminated soils and waters. Demonstrations of the biodegradation of polynuclear aromatic hydrocarbons by using a 30% (wt/vol) contaminated soil slurry have been reported (45). Other demonstrations have involved the treatment of explosive-contaminated soils. An aerobic reactor which used molasses as the cosubstrate reduced TNT from 1,300 to 10 mg/liter in 15 days (35); an anaerobic system achieved the same result more slowly. Other work indicated that the addition of a surfactant greatly enhanced the degradation rate of explosives by both acting as a cosubstrate and enhancing the bioavailability of the contaminants (85).

IN SITU APPLICATIONS

It has become clear that many nitroaromatic compounds can serve as growth substrates for bacteria. It is also evident that bacteria able to degrade nitroaromatic compounds are distributed in the environment at sites where contamination by nitroaromatic compounds has been chronic. Thus, it seems likely that treatment strategies based on in situ biodegradation of nitroaromatic compounds can be developed. Such treatment systems could avoid the necessity of providing alternate carbon and energy sources required by the treatment systems discussed in the preceding section. Many of the issues of materials handling and disposal of treated soils and effluents would also be minimized. Many in situ treatment systems for gasoline- and fuel-contaminated soils have been developed (6, 43) on the basis of the ready biodegradability of those compounds. With the discovery that bacteria can utilize many nitroaromatic compounds as growth substrates, it has become feasible to adapt many of the same treatment strategies to sites contaminated with nitroaromatic compounds. Compounds such as nitrobenzene, 3-nitrobenzoate, 2-nitrotoluene, 4-nitrotoluene, and 2,4-dinitrotoluene would be excellent candidates for in situ treatment.

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